Product Profile

HotStarTaq® *Plus* DNA Polymerase and Master Mix and HotStar HiFidelity Polymerase Kit

For highly specific hot-start PCR with minimal optimization

QIAGEN offers an unrivalled portfolio of highly specific and sensitive hot-start PCR products for a wide range of applications with minimal optimization required.

HotStarTaq *Plus* DNA Polymerase offers:

- Highly specific amplification — with minimal optimization required
- Fast 5-minute enzyme activation time — for increased speed
- Convenient procedure — with ready-to-load PCR buffer and room-temperature reaction setup

Highly specific and sensitive hot-start PCR

HotStarTaq *Plus* DNA Polymerase together with unique CoralLoad® or original PCR Buffer (provided) offers the same high specificity and unrivalled sensitivity as HotStarTaq DNA Polymerase, combined with an even faster 5-minute enzyme activation step. Amplification of nonspecific products, primer–dimer formation, and background smear are minimized in every PCR cycle (Figure 1). Highly specific results are obtained even when amplifying DNA from single cells (Figure 2).

Convenient kit format

The kit comes complete with enzyme, original PCR buffer, and readyto-load CoralLoad PCR Buffer — providing specific amplification with minimal optimization required (see page 2). Q-Solution®, a novel additive that enables efficient amplification of "difficult" (e.g., GC-rich) templates, is also provided.

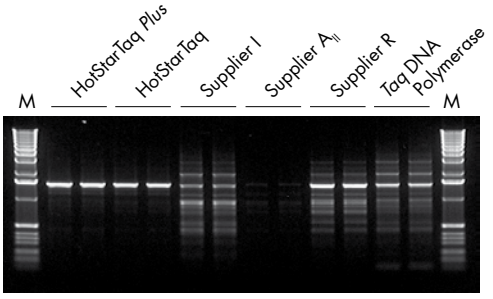

Figure 1. Highest specificity with HotStarTaq *Plus* DNA Polymerase. PCR was carried out using QIAGEN HotStarTaq *Plus*, HotStarTaq, and *Taq* DNA Polymerases and 3 hot-start PCR enzymes from the indicated suppliers. Parallel reactions were performed following the suppliers' recommendations, using 50 ng human genomic DNA. A 1.5 kb fragment of the human CFTR gene was amplified in 35 PCR cycles. Only QIAGEN hot-start enzymes provided highly specific amplification. **M**: markers.

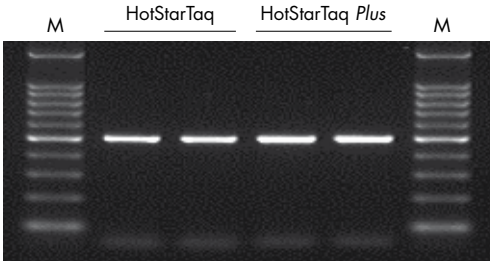

Figure 2. Highly sensitive single-cell PCR. Single-cell PCR of a 500 bp fragment of the murine p53 gene was carried out in duplicate using HotStarTaq and HotStarTaq *Plus* DNA Polymerase. Both enzymes showed equally high specificity and sensitivity. **M**: markers.

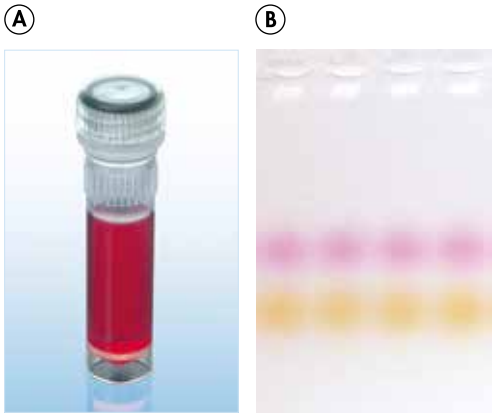

Figure 3. Convenient CoralLoad PCR Buffer and Concentrate. CoralLoad PCR Buffer and Concentrate contain two gel-tracking dyes enabling immediate gel loading of PCR products and easy visualization of DNA migration.

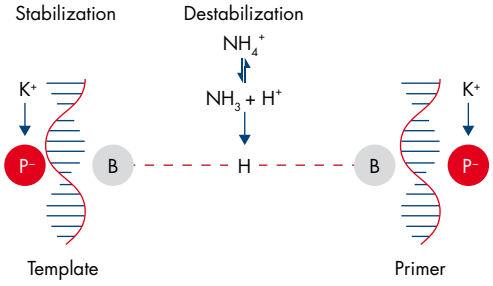

Figure 4. Effect of unique QIAGEN PCR Buffer. K^+ binds to the phosphate groups (**P**) on the DNA backbone, which stabilizes the annealing of the primers to the template. NH_4^+, which exists both as the ammonium ion and as ammonia under thermal-cycling conditions, can interact with the hydrogen bonds between the bases (**B**), destabilizing the weak hydrogen bonds at mismatched bases. The combined effect of the two cations maintains the high ratio of specific to non-specific primer-template binding over a wide temperature range.

HotStarTaq *Plus* Master Mix Kit

The HotStarTaq *Plus* Master Mix Kit provides all of the benefits of HotStarTaq *Plus* DNA Polymerase and more, including:

- Fewer pipetting steps — reducing the risk of contamination and pipetting error
- All components in one kit — including dNTPs

Fast, easy, and convenient PCR setup

The ready-to-use master mix contains HotStarTaq *Plus* DNA Polymerase, QIAGEN PCR Buffer, dNTPs, and $MgCl_2$. The kit is also supplied with CoralLoad Concentrate, which can be optionally added to the master mix prior to the start of PCR (Figure 3). With the HotStarTaq *Plus* Master Mix Kit, there is no need for separate pipetting of individual components, minimizing the risk of contamination. Lengthy calculations are avoided and setting up amplification reactions is fast and easy. Since errors due to pipetting variability and miscalculations are reduced, PCR results are highly reproducible. In addition, the hot start provided by HotStarTaq *Plus* DNA Polymerase in the HotStarTaq *Plus* Master Mix Kit leads to increased PCR specificity and amplification reactions can be set up at room temperature.

Minimal PCR optimization with unique PCR Buffer

QIAGEN PCR Buffer has been developed to eliminate the need for optimization of individual primer–template systems, saving time and money. The balanced combination of KCl and $(NH_4)SO_4$ in the buffer promotes specific primer–template annealing. Simultaneously, nonspecific annealing is reduced, maximizing yields of specific PCR product. Specific amplification is maintained over a wide range of temperatures and Mg^{2+} concentrations, without the need for time-consuming optimization (Figure 4).

Convenient ready-to-load PCR buffer

The novel CoralLoad PCR Buffer provided with HotStarTaq *Plus* DNA Polymerase and CoralLoad Concentrate provided with HotStarTaq *Plus* Master Mix contains two gel-tracking dyes, enabling immediate loading of your PCR product on to an agarose gel after amplification. Movement of the DNA on the gel can be easily monitored using the gel-tracking dyes (Figure 3).

HotStar HiFidelity Polymerase Kit

The HotStar HiFidelity Polymerase Kit provides:

- High sensitivity — due to novel buffer additive
- Direct cloning — in TA- or UA-cloning vectors
- High-fidelity amplification — tenfold higher than *Taq* DNA Polymerase

High sensitivity and reliability

HotStar HiFidelity DNA Polymerase is a hot-start proofreading enzyme uniquely modified to prevent degradation of primers and template during PCR setup, providing sensitive and specific high-fidelity PCR (Figure 5). The unique buffer system contains a balanced combination of K^+ and NH_4^+ ions (Figure 4) and includes Factor SB (patent pending), which greatly improves PCR sensitivity. The kit comes complete with enzyme and PCR buffer containing dNTPs and is ready to use with minimal optimization required. Q-Solution, a novel additive that enables efficient amplification of "difficult" (e.g., GC-rich) templates, is also provided.

Unique UA/TA cloning facility

In contrast to all other proofreading enzymes, HotStar HiFidelity Polymerase is uniquely modified to produce A overhangs, enabling streamlined and reliable UA/TA cloning (Figure 5). The cloning efficiency is comparable with fragments derived from *Taq* DNA polymerases, removing the need for less efficient blunt end cloning and timeconsuming verification sequencing.

Ideal for highly sensitive applications

The HotStar HiFidelity Polymerase Kit is ideal for all hot-start PCR applications that require high sensitivity combined with a low error rate, such as cloning and site-directed mutagenesis. The kit comes complete and ready to use with enzyme, PCR buffer (containing dNTPs), and Q-Solution.

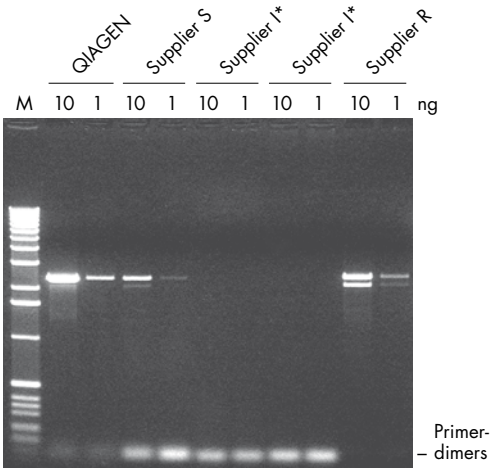

Figure 5. Highly sensitive and reliable PCR. PCR was carried out using HotStar HiFidelity DNA Polymerase (**QIAGEN**) and 4 high-fidelity PCR enzymes from the indicated suppliers. A 2.3 kb fragment of the human IL9R gene was amplified from differing amounts of genomic DNA in 40 PCR cycles. **M**: markers.

* Two different high-fidelity enzymes from Supplier I.

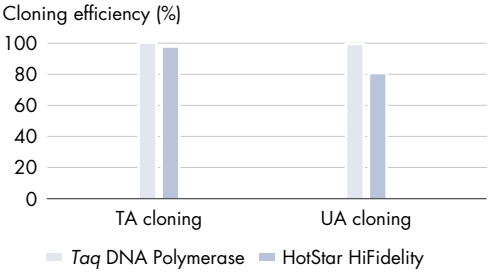

Figure 6. Efficient cloning with TA/UA cloning. A 955 bp PCR product was amplified from 100 ng of human genomic DNA using the HotStar HiFidelity Polymerase Kit and standard *Taq* DNA Polymerase. 4 µl of each PCR product was cloned using either a commercially available TA or UA cloning kit. Blue/white screening was used to determine the cloning efficiency.

Features of QIAGEN hot-start enzymes

	High specificity and sensitivity	Minimal optimization required	Fast 5-minute activation	High fidelity amplification	CoralLoad Buffer/ Concentrate for faster handling	Direct cloning in TA/UA vectors	Q-Solution for amplification of GC-rich templates
HotStarTaq *Plus* DNA Polymerase	Yes	Yes	Yes	–	Yes	Yes	Yes
HotStarTaq *Plus* Master Mix	Yes	Yes	Yes	–	Yes	Yes	–
HotStar HiFidelity Polymerase	Yes	Yes	Yes	Yes	–	Yes	Yes

Ordering Information

Product	Contents	Cat. no.
HotStarTaq *Plus* DNA Polymerase (250)	250 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer,* 10x CoralLoad PCR Buffer,* 5x Q-Solution, 25 mM $MgCl_2$	203603
HotStarTaq *Plus* DNA Polymerase (1000)	1000 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer,* 10x CoralLoad PCR Buffer,* 5x Q-Solution, 25 mM $MgCl_2$	203605
HotStarTaq *Plus* DNA Polymerase (5000)†	5000 units HotStarTaq *Plus* DNA Polymerase, 10x PCR Buffer,* 10x CoralLoad PCR Buffer,* 5x Q-Solution, 25 mM $MgCl_2$	203607
HotStarTaq *Plus* Master Mix Kit (250)	For 250 x 20 µl reactions: 2x HotStarTaq *Plus* Master Mix,‡ 10x CoralLoad Concentrate, RNase-Free Water	203643
HotStarTaq *Plus* Master Mix Kit (1000)†	For 1000 x 20 µl reactions: 2x HotStarTaq *Plus* Master Mix,‡ 10x CoralLoad Concentrate, RNase-Free Water	203645
HotStar HiFidelity Polymerase Kit (100)	100 units HotStar HiFidelity DNA Polymerase, 5x HotStar HiFidelity PCR Buffer (inc. dNTPs),§ 5x Q-Solution, 25 mM $MgSO_4$, RNase-Free Water	202602
HotStar HiFidelity Polymerase Kit (1000)	1000 units HotStar HiFidelity DNA Polymerase, 5x HotStar HiFidelity PCR Buffer (inc. dNTPs),§ 5x Q-Solution, 25 mM $MgSO_4$, RNase-Free Water	202605

* Contains 15 mM $MgCl_2$.

† Larger kit sizes available, please inquire.

‡ Contains 3 mM $MgCl_2$ and 400 µM each dNTP.

§ Contains Factor SB, dNTPs, and optimized concentration of $MgSO_4$.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Find out more about our highly-specific hot-start PCR products at **www.qiagen.com/goto/PCR**.